IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant (s): Navid Malik, Ruth Duncan, Donald A. Tomalia, and Roseita Esfand

Serial No.: 10/016,733 Group Art Unit: 1617

Filed: October 29, 2001 Examiner: Edward J. Webman

For: A DENDRITIC-ANTINEOPLASTIC DRUG DELIVERY SYSTEM

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION

STATE OF MICHIGAN)
) ss.
County of ISABELLA )

I, Donald A. Tomalia, declare and state:

THAT, I am an inventor of this application and an employee of Dendritic Nanotechnologies, Inc. (DNT), assignee of the above-identified application by an unrecorded assignment and hold the titles of President and Chief Technology Officer;

THAT, I am aware that the above-identified application is a continuation-in-part application of U. S. Serial No. 09/881,126, filed June 14, 2001 (now US Patent 6,790,437), which is a divisional of U. S. Serial No. 09/111,232, filed July 7, 1998 (now US Patent 6,585,956), which claims benefit of US Provisional Appln. No. 60/051,800 (now abandoned);

THAT, I am aware that the present application has an Official Action due, which I have read and reviewed, and in response to which this Declaration is being provided;

THAT, to overcome the objections raised, the following remarks are submitted;

THAT, the mechanism of binding cisplatin described in the original application in this series was on the surface of the dendrimer (see Figure 10) and that it was not yet fully

appreciated that the surface groups on the dendrimer could influence the encapsulation of cisplatin within the dendrimer;

THAT, this continuation-in-part application was filed when further data was available to demonstrate such encapsulation of the cisplatin;

THAT, under my supervision the following tests were run:

A. Determination of the loading of cisplatin on/in a dendrimer which data clearly demonstrated that less cisplatin was present than would be accounted for by the reaction of all the groups on the surface available to bind the cisplatin. See the results shown by Example 5A on page 22 lines 26-29. Stated another way, if the mechanism for binding cisplatin is as described in the original application in this series, with each cisplatin associated with one or two carboxylate surface groups, it would be expected that the surface would saturate at a number of cisplatins equal to the total surface groups. At G=3.5 there are 64 surface groups. Saturation would therefore covalently bond up to 64 cisplatin on the surface. Example 5A shows a range of cisplatin pickup of 25 wt % to a saturation of 40 wt%, which represents a cisplatin to dendrimer mole ratio of about 14/1 to 29/1, well below the expected maximum of 64/1 based on the previously proposed intermolecularly bonded structure.

B. Repeated washing of the cisplatin-dendrimer conjugate readily removes cisplatin from the interior of the dendrimer. See Example 12 on page 27. Thus it is apparent the cisplatin was interiorly chelated rather than covalently or irreversibly bound to the dendrimer. Exhaustive washing should not affect the nearly complete loss of covalently bound cisplatin as described. However, the intramolecularly (unimolecular) chelated cisplatin would provide rational support these losses.

C. The critical presence of the beta-amino carboxylate surface functional groups to assist shuttling the cisplatin to enter the first interior shell of the dendrimer is shown by Example 11, page 26, line 13 through page 27, line 5 where an amido or amidocarboxylate surface on the dendrimer was present resulting in lower cisplatin loading. This is in dramatic contrast when compared with Example 5A, page 22, lines 14-29, and Example 5B, page 23, lines 1-15, where an amino-carboxylate surface on the dendrimer was present resulting in higher loadings for both cisplatin and carboplatin (i.e. 25-40 wt% for cisplatin and 20.47 wt% for carboplatin).

D. I believe that the carboxylate surface on the dendrimer causes an ionic shunt mechanism to occur such that the cisplatin can be drawn into the first interior shell level, associated with the aminocarboxylate functionality. This leads to observed loading levels (i.e. 15-40 wt% as in Example 5A) that are rational for the proposed interior chelated structures. Given the similarity of chemical structures, it is reasonable to expect that carboplatin will behave similarly to the cisplatin molecule and form an analogous unimolecular conjugate with the dendrimer interior.

E. The AFM images of the dendrimer cisplatin conjugate clearly demonstrate monodispersed 4 nm diameter, unimolecular conjugate dimensions versus ill-defined larger dimensional structures that would be expected for crosslinked conjugates.

F. The platinum NMR spectra clearly supports the proposed amino-acid-platin interior chelate with a resonance band at 2120 ppm.

THAT, on July 22, 2003, I gave a talk to the 30th Annual Controlled Release Society Meeting in Glasgow, Scotland which in part illustrated the above remarks;

The enclosed Figures show the results of these experiments and that talk.

Figure 1: Shows the depicted location of the cisplatin by the red ovals which appear below the outer surface groups and reside in the first interior dendrimer shell.

Figure 2: States the evidence shown by the Examples in the present case where the surface groups influence the uptake of cisplatin by the dendrimer. Carboxylates must be geometrically related to the first shell of the amino groups as shown at the bottom of this slide to get substantial uptake. Please note that amino terminal groups at that generation lead to gels, much as proposed in the earlier patent, whereas converting such amino groups to acetamido groups or acetamido groups possessing carboxylate surfaces resulted in dendrimers which exhibited *no cisplatin uptake*. Therefore it has been demonstrated that carboxylate groups beta to the amino function in the outer shell are required to obtain the 15-23 wt% uptake observed at a reaction time of 24 hours and a wash time of less than 30 minutes as shown in Figure 2. These data support such intramolecular chelation versus covalent bonding at each terminal carboxy group or terminal bridging group.

Figure 3: Shows the actual AFM (Atomic Force Microscopy) images that provide unequivocal evidence that these G3.5 dendrimer cisplatin conjugates form discreet unimolecularly bound cisplatin as individual molecules with dimensions of approximately 4nm, which is close to the dimensional diameter of a 3.5 carboxylated dendrimer which has a diameter of approximately 4 nm, as determined by various methods. If our proposal was incorrect and bridging or crosslinking was dominating, one would expect to see background populations of larger sized clusters with higher diameters.

Figure 4: This slide shows our dendrimer cisplatin conjugate demonstrates a rational release profile that would be expected to be associated with a reversibly chelated unimolecular conjugate verses a covalently crosslinked or bridged conjugate.

Figure 5: The Figure 5 previously submitted with the last response shows the proposed dendrimer structure with cisplatin (Pt in red) where the cisplatin is NOT on the surface but below the outer layer as the Pt would associate with the teritary nitrogen and fold toward the interior. This proposed conjugate

structure accomodates the cisplatin uptake data, the reversible nature of the release profile data, the unimolecular dimensions (approximately 4 nm) as observed by AFM for individual, noncrosslinked structures, as well as the platinum NMR data shown on the next slide.

Figure 6: These Pt NMR data show resonance signals for our beta-aminoethylcarboxylate conjugate 2120 ppm are very close to those assigned for a similar aminocarbxylate conjugate described for a linear cisplatin polymer analogue reported by Access Pharmaceutical. Furthermore, our dendrimer cisplatin unimolecular conjugate shows the increased loading using the dendrimers as now claimed when compared with other linear HydroxyPropylMethacrylAmide (HPMA) polymers that bind cisplatin.

Thus, the carboxylated surface dendrimers dramatically increase the loading of the cisplatin (*i.e.* 20-25 wt% versus 7 wt%), when compare to other linear polymers or to amine surface dendrimers.

THAT, it was very unexpected to me that: (1) such mondispersed unimolecular conjugates as determined by AFM would be observed if it were crosslinked or bridged versus unimolecularly encapsulated; (2) such a smooth and moriotonic cisplatin release profile, which is similar to our other known dendrimer encapsulate release profiles, would be obtained if the conjugate were covalently crosslinked or bridged as described in the earlier patent; and (3) such a rational level of cisplatin loading for (e.g.15-23 wt%) would occur if the cisplatin were covalently bound exhaustively or bridged between dendrimer surfaces. Such rational cisplatin loadings and release profiles clearly support the proposed unimolecular encapsulation structure. Furthermore, the unexpected higher dose loadings of cisplatin in these proposed dendrimer conjugates offers a distinct advantage compared to analogous linear polymer conjugates. For some of these reasons this present continuation-in-part application was filed.

The undersigned DECLARANT declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Donald A. Tomalia

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Date: March 16, 2005
Encs. Figures 1 - 6